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POLLINATION BIOLOGY OF *DARLINGTONIA CALIFORNICA* (SARRACENIACEAE), THE CALIFORNIA PITCHER PLANT

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Abstract

The pollination ecology of *Darlingtonia californica* Torr., including especially the identity of its pollinators, has remained enigmatic for more than a century. The flowers of this well-known charismatic species are unusual in form and color, and have been the subject of much speculation. Accordingly, in this study we sought to identify *D. californica*'s floral visitors and determine their potential effectiveness as pollinators, in the context of *D. californica*'s unusual floral morphology. We also used hand-pollinations and emasculations to determine whether plants were pollen-limited at five study sites in northwest California, and to evaluate the potential for self-pollination in natural populations of *D. californic*a. A generalist solitary bee, *Andrena nigrihirta*, visited and pollinated *D. californica* flowers at five sites in northern CA. Despite very low visitation rates, individual flowers at all study sites were predicted to receive at least one visit by *A. nigrihirta*. Other regular floral visitors included thrips and several species of spiders. Plants at all five study populations were found to be pollen-limited with respect to the number of seeds produced per capsule. Fruit and seed production by emasculated flowers indicated a large degree of cross-pollination. However, emasculated flowers did not produce as many fruits and seeds as unmanipulated flowers, suggesting that self-pollination contributes to *D. californica* reproductive success as well. Observations of *A. nigrihirta* on flowers revealed that the shape and orientation of *D. californica*'s ovary and petals promote stigma contact both when pollinators enter and exit a flower, contrary to previous thought. Our findings provide evidence that *D. californica* is melittophilous, and suggest a resolution of the long-standing mystery surrounding the pollination of this rare species.

Key Words: *Andrena nigrihirta*, autogamy, *Darlingtonia californica*, pollination, xenogamy.

The only thing we are lacking is a pollinator.
(Schnell 1976)

The study of the interactions between plants and their pollinators can provide adaptive explanations for floral traits (Harder and Johnson 2009). Careful observation of flower patches —the essential first step in the process—usually generates a list of flower visitors, at least some of which are pollinators. Once the pollinators are known, adaptive hypotheses can be proposed based on an understanding of the biology of the animals as well as the ecological and the phylogenetic context. In spite of the crucial importance of "knowing the pollinators", the pollinator assemblages of a surprising number of plant species remain poorly or entirely unknown. The California pitcher plant, *Darlingtonia californica* Torr., is a case in point. The flowers of this well-known charismatic species are unusual in form and color, and have been the subject of much speculation (Debuhr 1973; Schnell 1976). For example, some have theorized that the bell-shaped ovary serves to limit self-pollination by directing pollinators away from the stigmas as they exit the flowers (Schnell 1976), but very few reports of pollinator visits exist despite serious interest from several workers (Austin 1875–1877 in Juniper et al. 1989; Elder 1997; Nyoka and Ferguson 1999; Nyoka 2000; Rice 2006). Published observations of flower handling by pollinators are lacking. In fact, the pollination ecology of this plant, including especially the identity of its pollinators, has remained enigmatic for more than a century.

The paucity of pollinator sightings is perplexing because fruit set in natural populations of *D. californica* is relatively high, and flowers do not self-pollinate autonomously (Elder 1997; Nyoka 2000). Based on appearances, the flowers of *D. californica* seem adapted for pollination by bees. They are large, showy, sweetly fragrant, and produce abundant pollen (Debuhr 1973; Nyoka and Ferguson 1999)—all features commonly associated with melittophily (Waser 2006). In addition, *D. californica*'s sister taxa, *Sarracenia* and *Heliamphora* spp., are pollinated predominantly by bumble bees (Thomas and Cameron 1986; Renner 1989; Ne'eman et al. 2006), suggesting that bee pollination may be primitive for Sarraceniaceae. Nevertheless, bees have seldom been observed as visitors to *D. californica* flowers (Austin 1875–1877 in Juniper et al. 1989; Elder 1997; Nyoka and Ferguson 1999; Nyoka 2000; Rice 2006). Spiders, in contrast, commonly

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use the flowers as hunting grounds (Austin 1875–1877 in Juniper et al. 1989; Elder 1997; Nyoka 2000). Although arachnids have generally not been given serious consideration as pollinators, Nyoka (2000) noted that they frequently constructed webs and stalked prey inside *D. californica* flowers and carried pollen on their bodies. By experimentally introducing spiders to bagged flowers, she showed that they can cause autogamy. She also found spiders carrying *D. californica* pollen outside of flowers, and detected fluorescent dye particles on spider draglines indicating their potential as cross-pollinators.

The hypothesis that the flowers of *D. californica* are pollinated by spiders is appealing, partly because it would explain why previous workers have seldom seen flying pollinators. However, spider pollination is problematic for at least two reasons. First, the effectiveness of spiders as pollinators may not be sufficient to account for observed levels of fruit and seed set. At her study site in southern Oregon, Nyoka (2000) found that fruit set of open-pollinated flowers approached 100%, and, on average, capsules produced more than 900 seeds. In contrast, fruit set of flowers bagged with spiders was less than 50%, and capsules produced 95% fewer seeds than open-pollinated flowers. This discrepancy implies that other visitors, perhaps bees, play a more important role as pollinators. Second, the morphological fit between spider and flower seems weak at best, making it difficult to conjure plausible adaptive explanations. In fact, the traits that create suitable conditions for spiders, such as the unusually long duration of anthesis (up to 48 days) and the protective tent-like corolla, may be adaptations for bee pollination. Occupation and occasional pollination of *D. californica* flowers by spiders may be incidental and secondary.

In spite of the long history of interest in the pollination of *D. californica*, to date there has been only one published account of a thorough, systematic survey for flower visitors (Nyoka 2000), making it premature to conclude that bees play little or no role in the pollination of the species. Here we report the results of extensive pollinator surveys at five sites in northwestern California. We combine these observations with results of experimental pollination treatments designed to estimate the degree of pollination limitation at these sites as well as the relative importance of self- vs. cross-pollination. The later should provide insight into the relative importance of spiders and bees as pollen vectors, assuming that spiders mainly cause self-pollination. We addressed four specific questions: (1) Who are the most important floral visitors, and are they capable of effecting pollination? (2) Is floral visitation by effective pollinators frequent in natural populations, i.e., is natural pollination sufficient, or are plants experiencing pollen-limitation? (3) Do cross-pollination and self-pollination each contribute to natural pollination? (4) Are past interpretations of the functional morphology of floral traits correct, i.e., does the ovary shape limit self-pollination as has been suggested (Schnell 1976)?

Methods

Study Species

Darlingtonia californica is a carnivorous plant endemic to western Oregon and northern California. Its distribution across this range is patchy, being restricted to perennial wet seeps, generally on serpentine soils (Juniper et al. 1989; Schnell 1976; Whittaker 1954). A long-lived perennial, *D. californica* produces rosettes of leaves from a creeping rhizome every year. Plants often occur in dense patches, which likely result from clonal spread by rhizomes and stolons (Schnell 1976).

The solitary flowers begin as upright buds, but become pendant when mature (Debuhr 1973). Unlike some *Sarracenia* (Ne'eman et al. 2006), the flowers of *D. californica* produce no nectar (Debuhr 1973). Abundant pollen is the only likely reward for pollinators, though a sugar-rich stigmatic exudate may also attract visitors (Nyoka 2000). Five lanceolate-ovate, yellow-green sepals hang loosely around five crimson petals. The five petals almost completely enclose the reproductive whorls, except for windows formed by notches in adjacent petals, which allow access to the flower's interior. The windows are level with the five stigmatic lobes, a feature that has been predicted to promote the deposition of outcrossed pollen as pollinators initially enter a flower (Schnell 1976). Twelve to fifteen stamens are located at the base of the ovary. The bell-shaped ovary is flared towards the stigmas, which has been postulated to function to guide pollinators away from the stigmas as they exit a flower and thus limit self-pollination (Schnell 1976). Flowers mature into upright capsules capable of producing around 2000 seeds (Debuhr 1973). The flowers of *D. californica* are self-compatible, but are not autonomously autogamous (Elder 1997; Nyoka 2000).

Study Sites

Five seeps, located near Scott Mountain and Mt. Eddy, CA were used in this study (Table 1). The five study sites will hereafter be referred to as SM1, SM2, CL, N17, and DF. Distance between sites ranged from ~0.1 to 14.5 km. Near the border of Trinity and Siskiyou counties, this portion of the Klamath Bioregion represents the center of *D. californica's* range (Debuhr 1973). Flowering occurred at all study populations

Table 1. Elevation and Geographic Coordinates of Five Study Sites.

Site	Elevation	Spatial coordinates
SM1	1635 m	41°16′25.00″N; 122°41′58.21″W
SM2	1630 m	41°16′38.57″N; 122°41′57.54″W
CL	1693 m	41°18′01.49″N; 122°40′59.90″W
N17	1945 m	41°20′08.05″N; 122°31′41.53″W
DF	2001 m	41°20′09.13″N; 122°31′11.39″W

between June 12, 2008 and June 22, 2008, except for CL where flowering started earlier (June 6, 2008). A total of 51 angiosperm species, all with blooming periods that at least partially overlapped that of *D. californica*, were present at the study sites (Meindl 2009). Within the study populations, common associates included white rushlily (*Hastingsia alba* S. Watson), California bog asphodel (*Narthecium californicum*Baker), Sierra shootingstar (*Dodecatheon jeffreyi* Van Houtte), marsh marigold (*Caltha leptosepala* DC *var. biflora* (DC) G. Lawson), and Bigelow's sneezeweed (*Helenium bigelovii* A. Gray in Torr.).

Flower Visitation and Pollinator Identification

Three observation points were established in each seep in order to monitor pollinator activity. At these points a series of 15-minute surveys were conducted, focusing on 13–17 flowers at one time. Ten surveys (2.5 hours total) were conducted during each day of observation at a field site. Each site was visited three to five times between June 6, 2008 and July 3, 2008 to conduct surveys. Most surveys were made between 10:00 a.m. and 6:00 p.m. In total, 57.5 hr of observations were conducted. Mean flower visitation rates (visits/flower/hour) and the estimated number of visits individual flowers would receive over their lifetimes were calculated for each study site (Meindl 2009). The expected number of visits a flower received over its lifetime was estimated by multiplying the flowering period (in days) by the number of hours in a day pollinators were active (six hr) by the visits/hour calculated for each site. *Darlingtonia californica* pollinators were considered to be active for six hours a day because all visits occurred between 10:30 a.m. and 4:30 p.m. Flower lifespan was determined by monitoring the development of 30 tagged buds at each study site (Meindl 2009).

Following each 15-min census period, five flowers were carefully examined by spreading apart the sepals and petals to check for pollinators already present within the flowers. A total of 1125 flowers were inspected in this way for spiders, spider webs (either inside or outside the flower), fungus gnats, and thrips. Insects were captured by aerial netting or by hand, and identified. For bees collected within the genus *Andrena*, individuals were identified using keys and descriptions from Laberge and Ribble (1975) and compared against previously identified reference specimens in the HSU invertebrate collection. Vouchers of all collected pollinators have been deposited at HSU for future reference.

Pollinator Behavior

To determine if floral visitors carried *D. californica* pollen, each insect collected during surveys (n = 88) was systematically dabbed with a small cube of glycerin jelly containing basic fuchsin stain (Kearns and Inouye 1993). Following pollen removal, the jelly was placed on a microscope slide, melted and covered with a cover slip for analysis. Pollen grains were identified by comparing them to a reference collection prepared from flowers at each site. *Darlingtonia californica* pollen was readily distinguishable from other pollen observed due to its unique morphology, which includes five elongate apertures extending from the grain walls. Pollinators were collected from the flowers of *D. californica*, as well as other coflowering species, to determine which members of the pollinator community carried *D. californica* pollen.

We could not determine the effectiveness of flower visitors directly, but instead recorded how often visitors gathered pollen and contacted stigmas, and how long they spent in flowers. A subset of observed floral visits was filmed with a digital camera. Along with other observed visits, the videos were analyzed to determine if pollinators handled the flowers in a manner that would result in pollination. These observations were also used to indicate whether or not the shape of *D. californica*'s ovary really serves to limit the occurrence of self-pollination.

Pollination Sufficiency and Estimates of Cross-Pollination

Hand-pollinations were performed to estimate pollination sufficiency. At each of the five study sites, 30 flowers were marked as controls and an additional 30 flowers were hand-pollinated. Supplemental pollen was applied twice (separated by one week) to flowers in the hand-pollinated treatment group by rubbing two-three mature anthers directly against stigmatic surfaces, when the appearance of stigmatic exudates indicated receptivity. Pollen used for hand-pollinations was collected from flowers at least five meters away in the same population. Fruit and seed set resulting from unmanipulated control flowers were compared against that of hand-pollinated flowers. If there is no difference in fruit set between these two treatment groups then we can conclude that natural pollination is sufficient, i.e., plants were not pollen-limited.

TABLE 2. FLORAL VISITATION RATES. The mean number of visits a flower was expected to receive per hour and over its lifetime is presented for each study site. Standard error values for mean visits/hour are given in parentheses for each study site.

Site	Visits/hour	Estimated visits/lifetime
CL	0.016 (SE = 0.029)	1.60
SM1	0.041 (SE = 0.042)	2.71
SM2	0.073 (SE = 0.025)	4.84
N17	0.077 (SE = 0.025)	5.08
DF	0.067 (SE = 0.025)	4.42

To gauge relative levels of cross-pollination vs. self-pollination, 30 flowers in each study population were emasculated prior to maturity. Fruits and seeds produced by flowers in the emasculated treatment group were interpreted to be the result of cross-pollination, whereas fruit and seed set by unmanipulated control flowers resulted from both cross-pollination and self-pollination. Thus the contribution of self-pollination to total pollination can be estimated by comparing the fruit and seed set of the emasculated flowers with the fruit and seed set of unmanipulated flowers.

A total of 450 flowers were used for fruit and seed set experiments, with 150 flowers in each of the three treatments: hand-pollinated, emasculated, and unmanipulated. These treatments were spread equally across the five study sites (i.e., 90 flowers at each site in 3 treatment groups of 30). Once fruit maturation began, all treatment flowers were bagged with Reemay® (Fiberweb, TN), a polyester fabric, to ensure seeds were not lost when capsules began to dehisce. Fruit set was determined for each site, as well as the number of seeds produced by each flower that matured a fruit.

Statistical Analyses

A Kruskal-Wallis one-way analysis of variance was used to compare average visitation rates across all sites. A non-parametric test was necessary to analyze visit rate data, as most data points were zeroes and thus the data set could not be adjusted to meet the assumption of normality. Log linear analysis was used to compare the fruit set of the three experimental treatment groups, with treatment, site and the interaction term included in the model. A two-way ANOVA was used to compare seed set across all sites, with treatment and site as the independent variables. Due to a significant interaction term from the two-way ANOVA ($P = 0.041$), separate one-way ANOVAs were run for each site independently using treatment as the independent variable. Post-hoc Tukey-Kramer multiple-comparison tests were used to determine which group means were significantly different from one another. All statistical analyses were performed using NCSS (Hintze 2004).

RESULTS

Flower Visitation and Pollinator Identification

In general, hymenopteran pollinators were abundant at our study sites, represented by eight genera of bees (Meindl 2009). However, *D. californica* received only 38 visits by flying pollinators in 57.5 hr of observations, and nearly all (37) were by a solitary bee, *Andrena nigrihirta*. One visit by a European honeybee (*Apis mellifera*) was also observed. Estimated visit rates varied widely (Table 2), but were not significantly different across the five sites (Kruskal-Wallis $\chi^2 = 5.72$, $P = 0.22$). Based on the average visit rate (pooled data across sites), flowers received 3.9 visits during their entire blooming period. Visits by *A. nigrihirta* were observed throughout the flowering season (6/13/09 through 6/22/09), and multiple visits were observed at each site (Meindl 2009).

Spiders, particularly members of the families Clubionidae, Salticidae, and Theridiidae, were common on flowers at all five study sites, and were active at all hours of the day (Table 3). Whereas a minority of examined flowers contained a spider, the majority showed evidence of spider occupancy (webbing and/or spider present)

TABLE 3. THE PERCENTAGE OF EXAMINED FLOWERS AT EACH STUDY SITE THAT CONTAINED ONE OR MORE OF THE FOLLOWING: THRIPS, SPIDERS, AND SPIDER WEBS (EITHER INSIDE OR OUTSIDE THE FLOWER). A total of 1125 flowers were individually examined (150 at CL, 225 at SM1, 250 at SM2, 250 at N17, and 250 at DF). "Evidence of Spider" column represents the percentage of examined flowers at each site that had a spider and/or webbing present. Only 3/1125 (0.27%) flowers contained one or more fungus gnats.

Site	Web outside flw.	Web inside flw.	Spider present	Evidence of spider	Thrips
CL	38.7	13.3	16.7	48.7	25.3
SM1	48.9	39.6	20	61.8	40.9
SM2	47.2	30.4	24.8	57.2	31.6
N17	69.6	20	34	74	75.6
DF	68.8	30.8	31.2	75.6	58.4
TOTAL	56.2	27.7	26.2	64.8	48.4

(Table 3). Thrips were also present in large numbers at all five sites: nearly half of all examined flowers contained thrips actively foraging for pollen (Table 3). Fungus gnats, while frequently encountered in the seeps, were only observed within *D. californica* flowers three times.

Pollinator Behavior

Individual bees spent up to several minutes within *D. californica* flowers and were found to carry *D. californica* pollen following visits. On average, *A. nigrihirta* foraged on a single *D. californica* flower for approximately two minutes and eight seconds (128 sec ± 12 seconds; n = 14). Eight individuals were collected immediately following visits, and all carried *D. californica* pollen in their scopae. Of these, six carried *D. californica* pollen exclusively while two carried heterospecific pollen as well (Asteraceae). One individual of *A. nigrihirta* was collected in flight (i.e., not on a flower) that carried both *D. californica* and Asteraceae pollen. *Andrena nigrihirta* was the only floral visitor collected that carried the pollen of *D. californica* (Meindl 2009).

Detailed observations of visits by *A. nigrihirta* revealed that the ovary shape of *D. californica* promotes stigma contact by bees both when they enter and exit flowers (Fig. 1). Immediately above the windows (towards the morphological base of the pendant flower), the flower's petals overlap and the underlying petal is appressed to the flared portion of the ovary, which limits the ability of a pollinator the size of *A. nigrihirta* to enter a window and crawl directly up onto the ovary on its way to collect pollen. In between the windows, however, the petals bulge outward (Fig. 2), and it is this space that allows the bee to ascend up to the stamens. This convex portion of each of the five petals is located directly opposite each of the five windows, such that a pollinator enters a window and walks in a straight line across the stigmas and then onto the ovary (directed by the convex portion of the petal). After ascending the ovary, bees were observed to systematically gather pollen before descending down the ovary towards the stigmas. The shape of *D. californica*'s ovary has previously been thought to guide an insect pollinator away from the receptive stigmatic surfaces as it exits the flower, thus preventing self-pollination. However, in exiting the flower, bees were observed to leave in the same fashion as they entered (guided by petal convexities across the stigmas and out one of the windows, thus likely effecting autogamy). This behavioral sequence was exhibited by multiple (n = 27) individuals and was consistent at all sites. These observations, plus evidence that *A. nigrihirta* carried the pollen of *D. californica*, strongly suggest these bees are acting as pollinators.

Pollination Sufficiency and Estimates of Cross-Pollination

Seed production, but not fruit set was pollen-limited. Fruit production by unmanipulated flowers (76%) was not significantly lower than that of hand-pollinated flowers (96%) ($\chi^2 = 3.50$, $P = 0.06$). However, hand-pollinated flowers produced more than twice as many seeds per capsule than unmanipulated flowers at each of the five study sites (Fig. 3).

Self- and cross-pollination both contribute to *D. californica* reproductive success. Emasculated flowers produced fruit and seed at all five sites, indicating that cross-pollination occurred. However, overall fruit set of emasculated flowers (39%) was significantly lower than that of unmanipulated flowers ($\chi^2 = 17.79$, $P < 0.001$), highlighting the importance of autogamous pollen transfer for fruit production. Unmanipulated flowers produced significantly more seeds, on average, than emasculated flowers at SM1 and SM2, but there was no significant difference found between these two treatment groups at the remaining three sites (Fig. 3). Average seed production by unmanipulated flowers was always higher than that of emasculated flowers, regardless of statistical significance, suggesting that cross-pollination cannot account for all of the seeds that were produced. Therefore, fruit and seed production of naturally pollinated flowers were likely the result of both autogamous and xenogamous pollen transfer.

Discussion

Near the summits of Scott Mountain and Mount Eddy in northwestern California, populations of *D. californica* are pollinated by the solitary bee *Andrena nigrihirta*, with additional pollination likely provided by spiders. This conclusion is based on direct observations of floral visits, analysis of bee pollen loads, and the results of our pollination treatments. In particular, even though visit rates were very low, we observed bee visits at all of our sites and estimate that flowers received an average of 4 visits over their extended blooming periods. We could not demonstrate directly that the bees deposited pollen on stigmas, but foragers consistently contacted stigmas when they visited flowers. Moreover, all captured individuals of *A. nigrihirta* carried *D. californica* pollen. Perhaps most importantly, nearly 40% of emasculated flowers produced fruits with seed sets equivalent to controls at three of the five sites. This result indicates substantial cross-pollination, and strongly implicates bees as pollen vectors. However, autogamy must have dominated at our sites because, with one exception (CL), fruit set of unmanipulated flowers was at least twice as high

FIG. 1. Step by step foraging behavior of *A. nigrihirta* on a *D. californica* flower. The bee initially lands on the petals below the windows (a–c) and then enters a window and walks across stigmatic surfaces (d, e). The bee then utilizes the convex portion of one of the five petals to walk onto the ovary and up to collect pollen (f–i). Following pollen collection, the bee uses a petal convexity as before to walk down the ovary, across the stigmas again, then out one of the windows (j–n), before leaving the flower (o, p). The flower is shown in d–m with the front petal removed and half of the two lateral petals removed. Panels f and k show the bee using the convex portion of the petal, which allows the bee to access the stamens.

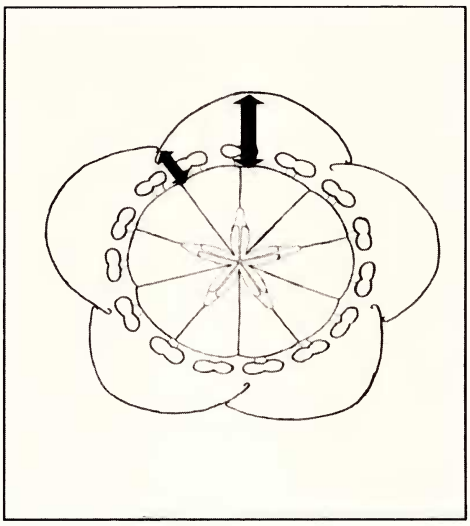

FIG. 2. Interior view of a *D. californica* flower with bottom portion of petals removed. Arrows highlight the distance between the petals and the ovary both immediately above a window (shorter arrow) and in between two adjacent windows (larger arrow). More space is provided for *A. nigrihirta* in between the windows than above them, which encourages the bee to enter a window and then walk across the stigmatic surfaces. The bee then utilizes the convex portion of the petal opposite the window it entered en route to the flower's stamens.

as fruit set of emasculated flowers (Fig. 3). Bees probably accounted for much of this self-pollination because they contacted stigmatic surfaces when they exited flowers after collecting large pollen loads. Spiders, which were abundant on flowers and known to be capable of effecting limited autogamy in *D. californica* (Nyoka 2000), likely further contributed to fruit and seed production via self-pollination. They often constructed webs inside flowers linking anthers and stigmas, and in several instances these webs were completely dusted with pollen. In contrast, spider draglines connecting flowers were very rare at our sites, making it unlikely that spiders contributed significantly to cross-pollination. Pollen-eating thrips were also present in large numbers within flowers, but were rarely seen on stigmas and thus likely played a limited role as pollen vectors. Nyoka and Ferguson (1999) collected fungus gnats carrying *D. californica* pollen in southwestern Oregon, where they may have contributed to seed set. However, although fungus gnats were abundant at our sites, we rarely discovered them inside flowers.

Our findings suggest a resolution of the long-standing mystery surrounding the pollination of *D. californi*ca. Like us, previous workers (Elder 1997; Nyoka 2000) reported high levels of fruit and seed production at their study sites in southwestern Oregon and the northern Sierra Nevada, but rarely or never observed flying pollinators – a discrepancy that led to the provocative hypothesis that omnipresent spiders are the most important pollinators. However, the high levels of pollen limitation observed in this study make it unlikely that spiders are the predominant pollen vectors for *D. californica*, given their abundance on flowers. Although spiders almost certainly contribute to pollen transfer in some degree, we propose instead that *D. californica* is melittophilous, as predicted by Schnell (1976), and specifically that *A. nigrihirta* is responsible for the majority of pollination across its range. Consistent with this view, we now know that *A. nigrihirta* pollinates *D. californica* in northwestern California as well as the northern Sierra Nevada (this study; Rice 2006). The same may be true for populations in southwestern Oregon, where Nyoka (2000) collected a pair of unidentified dark-bodied *Andrena* inside a flower. However, visit rates appear to be very low at all sites, which may partly explain why even observers who spent long periods in populations seldom observed visits. In addition, foragers tend to remain inside flowers for protracted periods (after quickly entering), and usually leave a population after visiting only one or two flowers (G. Meindl, unpublished). The difficulty of detecting these elusive bees is highlighted by the fact that although we spent well over 100 hours at our study sites setting up and monitoring experiments, we observed visits only during our focused census watches (10% of 230 watches). The alternative explanation for the limited number of previously reported visits is that *A. nigrihirta* was either absent or extremely rare at the sites studied by Austin, Elder, Nyoka, and Rice. Although spatial and temporal variation in the local abundance of bee species is well documented (Williams et al. 2001), this explanation begs the question of how to account for the high levels of fruit and seed production documented at these sites. A more parsimonious explanation may be infrequent but effective visits by *A. nigrihirta* coupled with the long period of anthesis of individual *D. californica* flowers. Clearly, additional timed surveys will be needed to document the relative abundance and importance of *A. nigrihirta* as a pollinator across the range of *D. californi*ca.

The relationship between *A. nigrihirta* and *D. californica* appears to be asymmetric, i.e., *D. californica* is specialized on *A. nigrihirta*, but *A. nigrihirta* is a generalist, at least on a broad scale. Across its range, which spans North America and greatly exceeds that of *D. californica*, *A. nigrihirta* is a generalist that has been observed to visit flowers from a diverse array of plants (Laberge and Ribble 1975), including members of Portulacaceae (Motten et al. 1982), Fabaceae (Tepedino et al. 1995), and Ericaceae (Rice 2006),

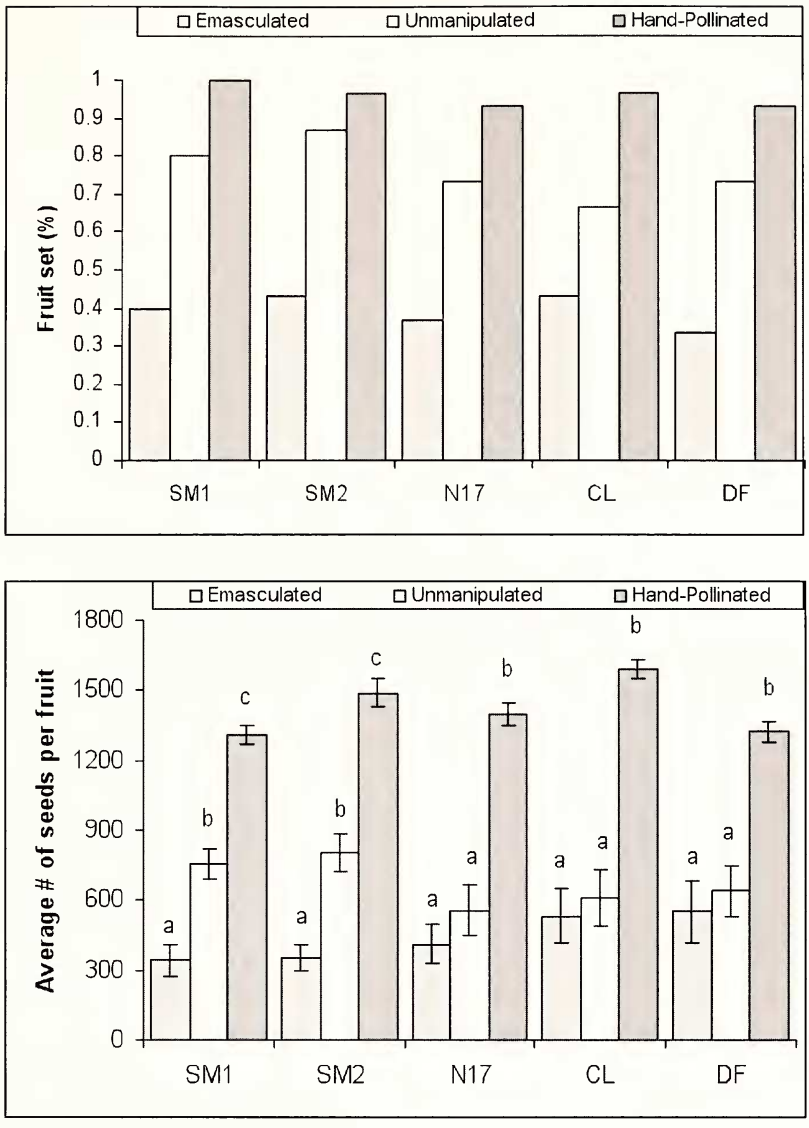

FIG. 3. Fruit and seed production by three treatment groups (emasculated, unmanipulated, and hand-pollinated flowers) at each study site. Top: fruit set (%) of three treatment groups at each field site. Emasculated flowers produced significantly fewer fruits than unmanipulated flowers. No significant difference was found between fruit production of unmanipulated vs. hand-pollinated flowers ($\chi^2 = 3.50$, $P = 0.06$). Bottom: average number of seeds produced per capsule from three treatment groups at each field site. Different letters above bars indicate group means are significantly different (comparisons of group means are only made within sites). Differences in mean seed production between the three treatment groups were evident at all 5 sites (SM1: $F_{2,63} = 65.35$, $P < 0.001$; SM2: $F_{2,65} = 53.76$, $P < 0.001$; CL: $F_{2,59} = 52.49$, $P < 0.001$; N17: $F_{2,58} = 46.31$, $P < 0.001$; DF: $F_{2,57} = 24.19$, $P < 0.001$).

along with *D. californi*ca. Three individuals of *A. nigrihirta* were collected during this study that carried both *D. californica* and Asteraceae pollen, indicating that *A. nigrihirta* is utilizing floral resources from multiple species of flowering plants. While asymmetrical species interactions are known to be common in ecological networks (Vazquez et al. 2007), it is unclear why *D. californica* relies so heavily on *A. nigrihirta* for pollination, considering the abundance of other bee species at our study sites.

Despite visits by *A. nigrihirta* being rare, the morphometric fit between bee and flower appears strong. While bumblebees were among the most abundant pollinators active at our study sites, their large body size prevented them from utilizing *D. californica* as a floral resource (G. Meindl, unpublished). Likewise, honeybees also have difficulty entering and handling the flowers (Rice 2006). *Andrena nigrihirta* was able to enter the small windows of *D. californica* flowers quickly and efficiently, and proved to be of an

ideal size to contact stigmas, climb onto the ovary beneath the petal convexities and gather pollen from the flower's anthers. Paradoxically, several other bee species collected at our field sites were of similar size to *A. nigrihirta* (e.g., other *Andrena* spp., *Osmia* spp., and *Lasioglossum* spp.; G. Meindl, unpublished), yet only *A. nigrihirta* was observed to forage on *D. californica* flowers. Further studies are needed to characterize the relationship between *D. californica* and its bee pollinators, and to determine why visits are made predominantly by *A. nigrihirta* and not by other similarly sized bee species. However, the preference shown to the flowers of *Darlingtonia* at our field sites in northern California, along with the morphological match between bee and flower, suggest that *A. nigrihirta* and *D. californica* have an established relationship. The detailed accounts of floral visitation in this study, combined with the results of pollination treatments, provide sound evidence that *D. californica* produces melittophilous flowers that are effectively, though rarely, pollinated by the solitary bee *A. nigrihirta*.

There are several interesting ecological questions that have yet to be considered regarding *D. californica* pollination. For instance, why are visits by bees so infrequent? How do spiders occupying *D. californica* flowers interact with bees? Does the presence of spiders within flowers deter visitation by bees, or do bees frequently fall victim to lurking spiders, and what bearing does this have on *D. californica* reproductive success? Over the course of floral observations conducted in this study, *A. nigrihirta* was seen "buzzing" flowers, i.e. approaching flowers but not entering them, more frequently than entering flowers (37 flowers visited, 50 flowers buzzed). While this "buzzing" behavior could be males searching flowers for females, other explanations are also possible. For example, this behavior could be the result of floral marking by bees, which may be done to alert future visitors of resource availability (Schmitt and Bertsch 1990; Goulson et al. 2001), or may also be the result of altered foraging behavior caused by the presence of flower-occupying spiders (Bruce et al. 2005; Goncalves-Souza 2008). It is also unclear how floral form influences pollination by bees vs. spiders, i.e., do the same floral traits that promote pollen deposition on stigmatic surfaces by bees (shape of ovary, etc.) also promote pollen deposition by spiders, or should we expect divergence of floral morphology in *D. californica* populations that occur in areas where *A. nigrihirta* is absent over time? As we seek to explain the adaptive significance of *D. californica*'s floral traits, we need to understand, in greater detail, the effects of these multi-species interactions on trait selection.

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Literature Cited

Bruce, M. J., A. M. Heiling, and M. E. Herberstein. 2005. Spider signals: are web decorations visible to birds and bees? Biology Letters 1:299–302.

Debuhr, L. E. 1973. Distribution and reproductive biology of *Darlingtonia californica*. M.S. thesis. Claremont Graduate School, Claremont, CA.

Elder, C. L. 1997. Reproductive biology of *Darlingtonia californica*. M.A. thesis. Humboldt State University, Arcata, CA.

Goncalves-Souza, T., P. M. Omena, J. C. Souza, and G. Q. Romero. 2008. Trait-mediated effects on flowers: artificial spiders deceive pollinators and decrease plant fitness. Ecology 89:2407–2413.

Goulson, D., J. W. Chapman, and W. O. H. Hughes. 2001. Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. Journal of Insect Behavior 14:669–678.

Harder, L. D. and S. D. Johnson. 2009. Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. New Phytologist 183:530–545.

Hintze, J. 2004. NCSS and PASS. Number Cruncher Statistical Systems, Kaysville, UT.

Juniper, B. E., R. J. Robbins, and D. M. Joel. 1989. The carnivorous plants. Academic Press, London, U.K.

Kearns, C. A. and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, CO.

Laberge, W. E. and D. W. Ribble. 1975. A revision of the genus *Andrena* of the western hemisphere. Part VII. Subgenus *Euandrena*. Transactions of the American Entomological Society 101:371–446.

Meindl, G. A. 2009. Pollination biology of *Darlingtonia californica*, the California pitcher plant. M.A. thesis. Humboldt State University, Arcata, CA.

Motten, A. F., D. R. Campbell, D. E. Alexander, and H. L. Miller. 1981. Pollination effectiveness of specialist and generalist visitors to a North Carolina population of *Claytonia virginica*. Ecology 62:1278–1287.

Ne'eman, C., G. Ne'eman, and A. M. Ellison. 2006. Limits to reproductive success of *Sarracenia purpurea* (Sarraceniaceae). American Journal of Botany 93:1660–1666.

Nyoka, S. E. 2000. The spider and the fly: a proposed pollination scenario for *Darlingtonia californica*, the California pitcher plant. Bulletin of the Native Plant Society of Oregon 33:68–69.

———— and C. Ferguson. 1999. Pollinators of *Darlingtonia californica* Torr., the California pitcher plant. Natural Areas Journal 19:386–391.

Renner, S. S. 1989. Floral biological observations on *Heliamphora tatei* (Sarraceniaceae) and other

plants from Cerro de la Neblina in Venezuela. Plant Systematics and Evolution 163:21–29.

RICE, B. 2006. Field trip report: *Darlingtonia* pollination studies in 2006. Website http://www.sarracenia.com/faq/faq7623.html [accessed 10 April 2011].

SCHMITT, U. AND A. BERTSCH. 1990. Do foraging bumblebees scent-mark food sources and does it matter? Oecologia 82:137–144.

SCHNELL, D. E. 1976. Carnivorous plants of the U.S. and Canada. John F. Blair, Winston-Salem, NC.

THOMAS, K. AND D. M. CAMERON. 1986. Pollination and fertilization in the pitcher plant *Sarracenia purpurea*. American Journal of Botany 73:678.

TEPEDINO, V. J., T. L. GRISWOLD, AND W. R. BOWLIN. 1995. Pollinator sharing by three sympatric milkvetches including the endangered species *Astragalus montii*. Great Basin Naturalist 55:19–25.

VAZQUEZ, D. P., C. J. MELIAN, N. M. WILLIAMS, N. BLUTHGEN, B. R. KRASNOV, AND R. POULIN. 2007. Species abundance and asymmetric interaction strength in ecological networks. Oikos 116:1120–1127.

WASER, N. M. 2006. Specialization and generalization in plant-pollinator interactions: a historical perspective. Pp. 3–17 *in* N. M. Waser and J. Ollerton (eds.), Plant-pollinator interactions: from generalization to specialization. The University of Chicago Press, Chicago, IL.

WHITTAKER, R. H. 1954. The ecology of serpentine soils IV. The vegetational response to serpentine soils. Ecology 35:275–288.

WILLIAMS, N. M., R. L. MINCKLEY, AND F. A. SILVEIRA. 2001. Variation in native bee faunas and its implications for detecting community changes. Conservation Ecology 5:7.